

CLAIMS

1. An electrochemical method for detecting the presence or absence of, or for determining the concentration of, NADH or NADPH in a sample comprising;

- contacting a reductase and a redox active agent with said sample; and
- 5 - measuring the quantity of reduced redox active agent produced by the reductase, by electrochemical means.

2. A method for monitoring the amount or activity of a redox enzyme or substrate, wherein the redox enzyme uses NADP, NADPH or NAD⁺ or NADP⁺ as a co-factor comprising carrying out the method of claim 1, wherein electron transfer between
10 the redox active agent and an electrode is correlated to the amount or activity of the redox enzyme or substrate.

3. A method according to claim 1 or 2, wherein the NADH or NADPH are produced by the reduction of NAD⁺ or NADP⁺ by a redox enzyme which concomitantly oxidises a substrate.

15 4. A method according to claim 3, wherein the amount of NADH or NADPH formed is proportional to the amount of the redox enzyme present or the amount of its substrate and hence allows the detection, or quantification of the enzyme or substrate in the sample.

20 5. A method according to claim 2, 3 or 4, wherein the redox enzyme is a dehydrogenase.

6. A method according to any one of the preceding claims wherein the reductase is capable of accepting two electrons from NADH or NADPH:

7. A method according to any one of the preceding claims wherein the reductase is selected from putidaredoxin reductase of the cytochrome P450_{cam} enzyme
25 system from *Pseudomonas putida*, the flavin (FAD/FMN) domain of the P450_{BM-3} enzyme from *Bacillus megaterium*, spinach ferredoxin reductase, rubredoxin reductase, adrenodoxin reductase, nitrate reductase, cytochrome *b₅* reductase, corn nitrate reductase, terpredoxin reductase and yeast, rat, rabbit and human NADPH cytochrome P450 reductase or a functional derivative of any thereof.

30 8. A method according to any one of the preceding claims wherein the redox active agent is Fe (CN)₆³⁻, Ru(NH₃)₆³⁺, or ferrocenium monocarboxylic acid (FMCA).

9. A method according to any one of the preceding claims wherein the reductase is specific for NADH.

10. A method according to claim 9 wherein the reductase is a putidaredoxin reductase.

5 11. A method according to any one of claims 1 to 8 wherein the reductase is specific for NADPH.

12. A method according to claim 11 wherein the reductase is the flavin domain of P450_{BM-3} or is spinach ferredoxin reductase.

10 13. A method according to any one of the preceding claims which allows a monitoring of the amount of the substrate, enzyme, NADH or NADPH over time.

14. A method according to any preceding claim wherein the redox active agent is not diaphorase or an organic dye.

15. An electrochemical cell comprising:

- sample holding means;
- a source of reductase;
- a redox active agent; and
- means for detecting and/or quantifying any current generated.

16. An electrochemical cell according to claim 15, which can be used to carry out a method as defined in any one of claims 1 to 14.